DEGRADATION OF PYRIDINES IN THE ENVIRONMENT

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I. INTRODUCTION

Pyridine and pyridine derivatives have recently received attention because of their presence in the environment and potential as a health threat.[1-3] These compounds occur naturally in the form of nicotinic acid derivatives and plant alkaloids,[4] and may enter the environment as the result of processing of synfuels and coal tars, pesticide application, and through a variety of chemical manufacturing activities. The ecotoxicological properties of pyridine have been reviewed recently.[5] The data presented suggested that pyridine itself need not be considered a large scale environmental pollutant. However, substituted pyridines occur more commonly than pyridine, and the chemical properties of pyridine are dramatically affected by the presence of minor substituents to the ring.[6] With the exceptions of a few pesticides, data are lacking on the fate of most substituted pyridines which have been detected in the environment. Despite limited knowledge of the environmental fate of pyridine derivatives, synthesis and utilization of this class of compounds have been increasing, and are expected to increase for the foreseeable future.[5] In light of occurrence of pyridines in surface and groundwaters, and the present trend toward commercial production of novel pyridine structures, a need clearly exists for a substantial database on the environmental fate of these compounds. The purpose of this article is to present a critical review of the information available on the occurrence and fate of pyridines in the environment, with emphasis on biological degradation of both naturally occurring and synthetic pyridine derivatives.

II. OCCURRENCE OF PYRIDINES IN THE ENVIRONMENT

Pyridine and pyridine derivatives are found throughout the environment at trace levels as components of biological systems. The pyridine ring generally occurs in nature as substituted pyridines, hydroxypyridines, pyridinones, and pyridine carboxylic acids. Pyridine, alkylpyridines, and chloropyridines are commonly of anthropogenic origin, and may be found at high levels in localized areas associated with industrial and agricultural activities.

A. NATURAL OCCURRENCE

Pyridine was originally extracted from bone oil by Anderson in 1849. Pyridine has been isolated from rayless goldenrod, *Aplopappus harwigi*,[7] but has not been observed in other plants. Fusaric acid (5-butylpicolinic acid), fusarinin (3-butylpyridine), and picolonic acid (pyridine-2-carboxylic acid) were produced by several plant pathogens.[8-10] Dipicolonic acid (pyridine-2,6-dicarboxylic acid) was found in bacterial endospores accounting for 7 to 13% of the weight.[11] Plant alkaloids of the pyridine-pyrrolidine class, including nicotine, nornicotine, and anabasine, have been found in a number of species of *Nicotiana*.[4] Quinoline and isoquinoline derivatives have also been found as components of plant alkaloids.

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Though pyridines are present only at trace levels in most biological systems, they are important components of many metabolic pathways. Two pyridines common to all biological systems are nicotinamide and pyridoxine (vitamin B_6). Nicotinamide is necessary for the synthesis of the coenzymes nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). The nicotinamide coenzymes, as carriers of reducing equivalents, are essential to a wide range of catabolic and anabolic cellular processes. Pyridoxine and the related compounds pyridoxal and pyridoxamine, form the coenzyme pyridoxal-5′-phosphate, which is involved in reactions governing the metabolism of α-amino acids.

Pyridines have been identified in many food products including vegetables (artichokes,[12] asparagus,[13] beans,[14] tomatoes,[15] meat products,[16-18] cheese,[19] eggs,[20] pecans,[21] peanuts,[22] cocoa,[23] and coffee.[2] However, few of the pyridines which have been identified are found to be naturally occurring. Most of the foods in which pyridines have been identified have undergone some form of thermal treatment or microbial fermentation.[25] Pyridine was evolved during the putrification of the molusk *Mytilus edulis*, however it was not detected in fresh mussel, suggesting that it was produced through the action of microorganisms.[26] Pyridine has also been identified in the volatile component of mango preserved by deep freezing, but was not found in fresh mango.[27] Pyridines have been identified in roasted beef[18] and in the basic fraction of steam volatile oil from potato chips,[28] suggesting that they contribute to the characteristic aromas of these foods. It is likely that *in situ* pyrolytic reactions during food processing produce most of the pyridines found in food. Thermal decomposition of the amino acid A-alanine was found to produce 2-methyl-5-ethylpyridine,[29] while cysteine formed pyridine, 2-methylpyridine, and 3-methylpyridine in the presence of glucose.[30]

B. ANTHROPOGENIC INPUTS

Greater attention has been focused on the occurrence of pyridines in the environment as the product of industrial activities. The technologies used in the production of synthetic fossil fuels have been associated with contamination of aquatic and terrestrial environments with polycyclic aromatic hydrocarbons (PAHs) and *N*-heterocyclic aromatic compounds, many of which are produced through pyrolytic reactions during coal gasification and oil shale retorting. Pyridines, and other azaarenes including quinoline and acridine, have been found in coal liquification products and shale oil retort water.[31] These processes present a novel environmental challenge since the products and waste waters produced in synthetic fossil fuel processing are chemically different from the wastes associated with petroleum production. Since an estimated 50% of the U.S. population relies upon ground water for commercial and municipal purposes,[32] there is great concern over the impact that large scale development of synthetic fossil fuel technologies will have on local ground water resources. Pyridines may also enter the environment as a consequence of their use as insecticides and herbicides in agriculture, or through industrial activities associated with pharmaceutical manufacture and chemical synthesis.

A major environmental concern associated with underground coal gasification (UCG) processes is the potential for local ground water contamination through leaching of the residual ash which remains in the gasification cavity and by volatile organic compounds which escape into the surrounding underground formation.[2] Contaminants identified in the basic fraction of ground water taken near a UCG site in northeastern Wyoming consisted mainly of pyridine, 2, 3-, and 4-methylpyridine, other alkylpyridines, quinoline and isoquinoline, and alkylquinolines at μg/l levels.[2] The aqueous phase of ground water from a shallow aquifer at the site of a coal-tar distillation site in St. Louis Park, Minnesota was found to contain 2-, 3-, and 4-methylpyridine, 2- and 4-dimethylpyridine, quinoline and a suite of alkylquinoline derivatives, and acridine.[32] The presence of acridine was of particular concern owing to its toxicity and its persistence in aquatic environments. Ground water near the site of a wood treatment-coal tar processing plant in Pensacola, FL was contaminated with quinoline (the major base in coal tar)

and isoquinoline, and high levels of the oxygenated analogs 2(1H)quinolinone and 1(2H)isoquinolinone.[33] The enhanced levels of the oxygenated derivatives are consistent with their high solubility and therefore their mobility in ground water. The oxygenated analogs have not been previously identified in wood preservative waste water, suggesting that they are products of indigenous microbial activity.[32] The current data base clearly suggests that organic bases, particularly pyridines and quinolines, which were found in coal-tar wastes in contact with groundwater can leach into the aqueous phase.[32] In addition, coal-tar derivatives have been found to enter ground water from above-ground sources by percolation through the unsaturated zone or by infiltration from uncontained waste water basins above the water table.[34]

During the retorting of shale oil, water vapor at high temperatures and under pressure was held in close contact with the oil and the solid matrix, which resulted in the production of retort water contaminated with a large number of organic and inorganic compounds.[35] Shale oil production by present *in situ* processes generates equivalent volumes of process waste waters which are highly contaminated with organic solutes. During shale oil recovery, large volumes of these waste waters are brought to the surface. The waters are separated from the oil, stored in tanks and holding ponds, and eventually treated. At any point in the process the possibility of spillage exists. Large spills, or leaching or seepage from holding ponds, pose the threat of contamination of surface water or shallow ground water supplies.[36] Alkylpyridines have been identified in water from one of several alluvial wells and in a surface seep located near a retorted shale disposal pile in Rifle, CO.[37] Total alkylpyridine concentrations were 30.8 and 4.1 μg/l, respectively. Lower concentrations were found in stream waters sampled below the seep (0.1 to 0.2 μg/l), reflecting a dilution of the seep discharge.[37] The base fraction from an extraction of oil shale retort water from Rundle, Australia, consisted almost exclusively of *N*-heterocyclic compounds.[35] Pyridine, 2-, 3-, and 4-methylpyridine, 2,5-dimethylpyridine, 2,4,6-trimethylpyridine, 2- and 4-ethylpyridine, other alkylpyridines, quinoline, isoquinoline, and several alkylated quinolines were present at mg/l concentrations. It has been suggested that alkylpyridines[37] may be useful diagnostic indicators of the contamination of waters by oil shale retorting since these compounds have not been found in aqueous petroleum extracts and are commonly found in conjunction with synthetic fossil fuel processing.

A comparison of the organic contaminants of a shale oil retort water with a gas condensate retort water produced during *in situ* retorting at the Occidental Oil Shale facility at Logan Wash, CO indicated that dissolved organics in the condensate water were mainly steam-volatile polar compounds, whereas retort water contained nonvolatile organic anions and polyfunctional neutral compounds.[38] Contaminants in the base fractions of gas condensate consisted almost exclusively of *N*-heterocyclic compounds. The base fraction of the process water contained high levels of hydroxypyridines and trace levels of pyridinecarboxylic acids. The hydroxypyridines included 2-, 3-, and 4-hydroxypyridine and 2-hydroxy-6-methylpyridine with a range of concentrations from 25.6 to 4.0 mg/l. Pyridine carboxylic acids were present at a concentration range from 0.5 to 0.3 mg/l and included all three mono-substituted isomers. Volatile forms such as pyridine and alkylpyridines were present only at trace levels. The base fraction of the condensate water contained higher levels of pyridine, 2-, 3-, and 4-methylpyridine 2,6-dimethylpyridine, 2,4,6-trimethylpyridine, quinoline, and isoquinoline ranging in concentration from 70.1 to 1.6 mg/l. Hydroxypyridines and pyridinecarboxylic acids were not detected in the condensate water, which was predicted by the low volatility of these compounds.

There has been concern that the volatile components of retort wastewater could pose a localized hazard to air quality. This was particularly true for shales in the Western United States, where shale oil wastewaters were generally characterized by a pH of 8 to 10,[39] which favored the neutral species of pyridine and alkylpyridines and thus enhanced volatility. A study of the emission of air pollutants from shale oil wastewaters[39] indicated that significant levels of 2,4- and 2,6-dimethylpyridine, 2,4,6-trimethylpyridine, and C_3 and C_4 alkylpyridine isomers were found in the air above shale oil wastewaters. Purge and trap analysis of these same waters

indicated the presence of pyridine, 2-, 3-, and 4-methylpyridine, 2,3-, 2,4-, and 2,6-dimethylpyridine, 2,3,4-trimethylpyridine, 2-ethylpyridine, various C_3, C_4, and C_5 alkylpyridine isomers, and quinoline. However, it was projected that detectable levels would be localized at site areas, posing minimal danger to the surrounding environment.

Pyridine and pyridine derivatives have been used extensively in industry as solvents and for the synthesis of a wide range of compounds used in agriculture, textiles, pharmaceuticals, and industrial manufacture. Halogenated pyridines have been used as organic intermediates in the production and systhesis of many agrochemicals. Picloram (4-amino-3,5,6-trichloropicolinic acid), nitrapyrin (2-chloro-6-[trichloromethyl] pyridine), fluridone (1-methyl-[3-phenyl-5-(triflouromethyl)phenyl]-4(1H)-pyridinone), and chloropyrifos (3,5,6-trichloro-2-pyridinol) are several examples of this class. Widespread use of such herbicides and insecticides can result in low level contamination of surface and ground waters in areas in and around the zone of application.[40] Pyridine is used in the manufacture of the desiccant herbicides paraquat (1,1′-dimethyl-4-4′-bipyridinum ion) and diquat (6,7-dihydropyrido [1,2-A:2′,1′-C] pyrazinediium ion).

Pharmaceutical agents include isoniazid, a therapeutic agent used in the treatment of tuberculosis, cetyl pyridinum bromide (a detergent), the respiratory stimulant coramine, α- and β-eucaine (local anesthetics), and the analgesic demerol.[41] A number of antihistamines including chlorpheniramine maleate and pyrilamine maleate contain the pyridine moiety. Various coal-tar products used in the treatment of psoriasis and severe eczema have been found to contain quinoline.[42] Several pyridines have found use as commercial food additives for flavor and aroma enhancement. Generally they are added to produce a concentration in food in the mg/kg to μg/kg range. Among the compounds used are pyridine, acetylpyridines, several alkylpyridines, and quinoline.[25]

III. ECOTOXICOLOGY

A. PROCARYOTIC MICROORGANISMS

The Ames bacterial mutagenicity assay, which employs histidine-auxotrophic mutants of *Salmonella typhimurium,* has been used extensively for the purpose of screening compounds for mutagenic potential. Ames testing of shale- and coal-derived oils has found that most of the mutagenic activity was associated with the basic and neutral fractions.[43] These fractions contain an array of polycyclic compounds, such as the azaarenes, quinoline, acridine, benzoquinoline, and their alkyl derivatives. However, based on screening with the Ames assay, azaarenes having 2 to 4 rings were relatively minor contributors to the overall mutagenic potential of synfuel products,[44] though quinoline is significantly more mutagenic than pyridine.[45] Pyridine and piperidine were both tested negative for mutagenic activity by both the Ames *S. typhimurium*/microsome assay and an *Eschericia coli polA*$^+$/ *polA*$^-$ assay.[46] The chlorinated pyridines 2-chloromethylpyridine HCl, 3-chloromethylpyridine HCl, and 2-chloro-6-trichloromethylpyridine have shown mutagenic activity in *Salmonella*.[47] The mutagenic activity of 2-chloropyridine was observed only when subject to microsomal activation, suggesting that the *N*-oxide is the mutagenically active form. One pyridine derivative has demonstrated a specific toxic activity in nitrifying bacteria. Two-chloro-6-trichloromethylpyridine (*N*-serve) has been used commercially as a nitrification inhibitor in soil.[4]

B. EUCARYOTIC MICROORGANISMS

Numerous studies of the structure activity relationships among pyridines and other *N*-heterocyclic compounds have been conducted using the cilliated protozoan *Tetrahymena pyriformis* as the test organism.[48-52] *Tetrahymena* has widespread distribution in nature and is considered representative of microfauna of aquatic ecosystems, providing an index of a healthy aquatic environment. It is considered an ideal organism for toxicity testing in aquatic systems

TABLE 1
Growth Impairment to *Tetrahymena* and Physiochemical Descriptors of Selected *N*-Heterocyclic Compounds

Compound	60-h IGC_{50} μM	log KOW[a]	Number of rings	Number of in-ring nitrogen atoms
Pyrazine[c]	66.360	–0.22	1	2
Pyrimidine[c]	56.500	–0.40	1	2
4-Hydroxypyridine[b]	38.634		1	
Pyridazine[c]	25.510	–0.72	1	2
4-pyridinecarboxylic acid[b]	23.819		1	
Pyridine[e]	15.320	0.64	1	1
4-Methylpyridine[b]	7.850		1	
4-Chloropyridine.HCl[b]	7.275		1	
2-Aminopyridine[d]	4.175	0.52	1	1
4-Aminopyridine[d]	2.750	0.27	1	1
Quinoxaline[c]	2.010	1.10	2	2
4-Ethylpyridine[b]	1.981		1	
Quinazoline[c]	1.950	0.92	2	2
5-Aminoquinoline[d]	1.640	1.27	2	1
4-Pyridinecarboxaldehyde[b]	1.442		1	
3-Aminoquinoline[d]	1.177	1.51	2	1
Quinoline[a]	0.970	2.04	2	1
5-Nitroquinoline[d]	0.409	1.87	2	1
4-Nitropyridine[d]	0.390	0.60	1	1
6-Nitroquinoline[d]	0.346	1.94	2	1
4-Phenylpyridine[b]	0.217		1	
4-Vinylpyridine[b]	0.086		1	
Acridine[e]	0.040	3.40	3	1

[a] K_{ow} = octanol-water partition coefficient.
[b] From Schultz and Moulton.[52]
[c] From Schultz and Cajina-Quezada.[49]
[d] From Schultz and Applehans.[51]
[e] From Schultz et. al.[48]

due to the ease and inexpense of culture, its well-researched biology, and its rapid response to environmental stress; a result of the lack of a sophisticated homeostatic mechanism.[50] Though a number of biological responses are monitored in toxicological assays, among the most sensitive of sublethal responses is decreased reproductive ability, which is quantitatively expressed as IGC_{50} (the concentration which results in 50% growth inhibition as compared to control populations). Table 1 shows various pyridines and other azaarenes in order of increasing toxicity to *Tetrahymena*. Growth impairment increased with an increase in the number of aromatic rings per compound and with crowding of nitrogen within the ring, though the addition of in-ring nitrogen resulted in an overall decrease in toxicity. The correlation between growth impairment and log K_{ow} (1-octanol/water partition coefficient) was taken as an indication that the rate limiting step was passive cellular uptake,[50] since compounds with higher log K_{ow} values have a greater affinity for lipids and may thus more readily penetrate lipid containing cell membranes.[53] It was proposed by Schultz and Cajina-Quezada,[49] that the common mechanism of action causing the impairment of population growth may have been due to the effects of conformational changes in the membrane on membrane bound proteins, resulting from the partitioning of compounds in the lipid region of the membrane. The comparatively high toxicities of 4-vinylpyridine and 4-nitropyridine were due to specific toxic mechanisms, the former alkylates sulfhydryl groups and the latter can form the highly toxic nitroxy free radical.

In a study on the chronic toxicity of oil shale retort waters, *Nitzschia closterium*, a common Great Barrier Reef diatom, was cultured in medium containing a sublethal dose of retort water for 14 weeks.[54] Quinoline was found to be of moderate toxicity while pyridine and 2-hydroxypyridine enhanced algal growth at the concentrations found in retort water (quinoline 2(0.9-11), pyridine 5(4-50), and 2-hydroxypyridine 14×10^{-5} *M*, range of published values in parenthesis. A related diatom *N. palea* was found to have a 4-h EC_{50} (a concentration causing a 50% reduction in the rate of ^{14}C assimilation) of 104 mg/l for quinoline and 20.8 mg/l for acridine while the green algae *Selenastrum capricornutum* had 4-h EC_{50} values of 202 and 20.0 mg/l, respectively.[53] Pyridine has been found to exhibit toxic effects in *Scendesmus quadricuada* (a green algae) at a concentration as low as 120 mg/l.[55]

C. INVERTEBRATES

Pyridine toxicity has been found to vary widely among invertebrate species. In a study of 12 macroinvertebrates,[56] 48-h LC_{50} values ranged from 30 mg/l for the amphipod *Gammarus pulex* to 2400 mg/l for *Erpobdella octoculata*, a hirudinean (leech), with intermediate values among *Dugesia* cf. *lugubris* (planarian) 1900 mg/l, *Tubificidae* (various species) 1300 mg/l, *Hydra oligactis* 1150 mg/l, *Ischnura elegans* (damselfly) 410 mg/l, *Lymnaea stagnalis* (snail) 350 mg/l, *Nemoura cinerea* (stonefly) 254 mg/l, *Chironomus gr. thummi* (midge) 229 mg/l, the crustaceans *Asellus aquaticis* 220 mg/l and *Gammarus pulex* 182 mg/l, and *Cloeon dipterum* 165 mg/l. Quinoline was found to affect embryogenesis and hatching success in the common snail *Physa gyrina* at concentrations as low as 25 mg/l, which is significantly lower than the 48-h LC_{50} of 183 mg/l.[57] A strong correlation between the toxicity of *N*-heterocycles and the number of rings has been demonstrated with several invertebrate species, acridine having had an order of magnitude lower 48-h LC_{50} than quinoline.[53,56]

Several pyridine derivatives are highly toxic to certain insect pests and have thus found use as insecticides. The naturally occurring plant alkaloids nicotine and related nornicotine and anabasine, have demonstrated toxic activities in aphids and certain species of mites and ticks.[4] The commercially synthesized insecticide chloropyrifos, a moderately toxic broad spectrum insecticide, has enjoyed extensive use as a mosquito larvicide and for control of chinch bugs and white grubs in lawns.

D. VERTEBRATES

Pyridines exhibit a range of toxic activities among vertebrates. Pyridine acute oral LD_{50} values for rat, mouse, and guinea pig have been recorded at 1500, 1500, and 4000 mg/kg, respectively.[58,59] In man, 500 mg/kg (oral) has been the lowest published lethal dose LDL_0 for pyridine.[59] Among mammals, subacute toxicity responses have included gastrointestinal disorders and kidney and liver damage.[5] The toxic activities of pyridines have been found to vary from compound to compound. Acute oral toxicities (LD_{50}) for the herbicide picloram were relatively low, ranging from 2000 mg/kg for rabbits to 8000 mg/kg for rats, while nicotine was highly toxic, with a rat acute oral (RAO) LD_{50} of 30 mg/kg.[4] In mammalian systems, *N*-heterocyclic compounds have been found to undergo metabolic reactions at both the nitrogen heteroatom (*N*-methylation and *N*-oxidation) and at the ring carbons (*C*-oxidation). In a study in which *in vivo* metabolism of pyridine was investigated in the rat, hamster, mouse, gerbil, rabbit, guinea pig, cat, and man,[60] most of the species produced pyridine *N*-oxide, *N*-methyl pyridinum ion, 2-pyridine, 3-hydroxypyridine, and 4-pyridone as metabolites, however the proportion of the dose excreted as each of these metabolites was species dependent.

As with microorganisms and invertebrates, the toxic activity of *N*-heterocyclics has been found to increase with increasing ring number, which is directly correlated with an increase in K_{ow}. In a study of largemouth bass (*Micropterus salmoides*) and rainbow trout (*Salmo gairdneri*) eggs and larve,[53] acridine was found to be significantly more toxic than quinoline. Seven-day LC_{50} values for largemouth bass were 7.42 mg/l for quinoline and 0.91 mg/l for acridine, while

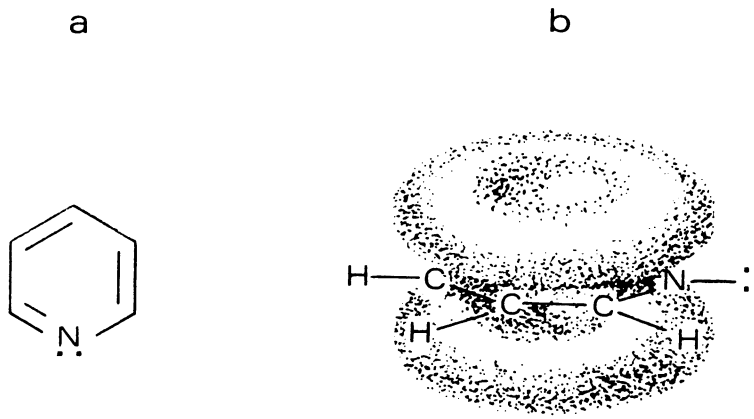

FIGURE 1. Kekule' structure for pyridine (a). Overlapping of p orbitals forms pi clouds above and below the ring (b). Note lone pair of electrons in sp^2 orbital of nitrogen (a and b). (Figure 1b adapted from Morrison, R. T. and Boyd, R. N., *Organic Chemistry*, 4th ed., Allyn and Bacon, Boston, 1983, 1267.)

27-d LC_{50} values for rainbow trout were 11.5 and 0.30 mg/l, respectively. A similar response was seen in the same test organisms in a study by Black et al.,[61] where acridine exhibited a stronger teratogenic response than quinoline.

E. PLANTS

Recognition of the phytotoxic activities of pyridine Á-carboxylic acids has lead to their use as herbicides. Four-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram) has been used extensively for control of broadleaf weeds and brush. Picloram has been found to exert auxin-like effects on plants, in a manner similar to fusaric acid (5-butyl-2-pyridinecarboxylic acid) produced by the plant pathogen *Fusarium lycopersici*, though the mechanism is not known. Chang and Foy[62] found that while picloram and other pyridine Á-carboxylic acids were able to form complexes with metal ions, their toxic action in plants was not likely due to their depletion of free metal ions, nor the inhibition of auxin oxidase through strong chelation of the Fe-porphyrine moiety.

F. CONCLUSIONS

In general, pyridine is not highly toxic for most organisms. However, slight modification of ring substituents can dramatically affect the toxic activity of pyridines. The toxicity of pyridines varies not only from compound to compound, but also among species; with some pyridines having a highly species-specific activity. Though there is a wide range in the toxic activities of azaarenes, there is, with few exceptions, an increase in the toxic response of a given organism to these compounds as the number of rings increases.

IV. CHEMICAL PROPERTIES OF PYRIDINES

Pyridine is a six-membered aromatic heterocycle with nitrogen as the sole heteroatom (Figure 1a). It is a planer molecule with average bond angles of 120°. The compound differs from other *N*-heterocyclics, such as pyrrole, in that the third sp^2 orbital of the nitrogen atom in pyridine contains only a pair of electrons (Figure 1b), which in pyrrole is involved in the pi cloud. For this reason, pyridine differs from pyrrole in the availability of the nitrogen heteroatom to share electrons with acids (Figure 2), and is therefore a much stronger base than pyrrole. However, in aqueous solutions, pyridine is a much weaker base (pK_a = 5.25) than aliphatic amines (pK_a for ammonia = 9.26, for 4-aminobutyrate = 10.56).[63]

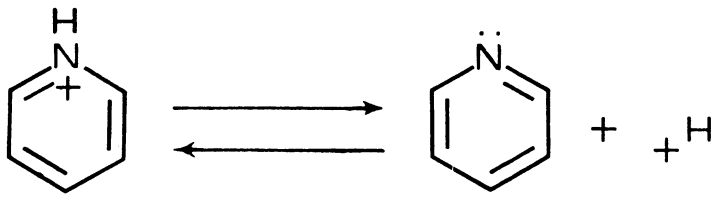

FIGURE 2. Basicity of pyridine. Note formation of pyridinium cation (pKa + 5.25).

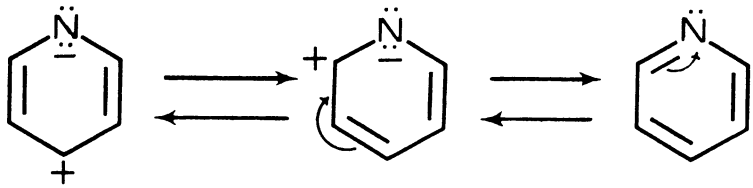

FIGURE 3. Resonance structures for pyridine. Note deficiency of electrons at positions 2, 4, and 6.

Because of the electronegativity of the nitrogen atom, the ring tends to be electron deficient, particularly at positions ortho and para to the nitrogen, while the nitrogen atom tends to electron rich (Figure 3). The unusual electronic structure of pyridine accounts for the somewhat uncharacteristic behavior of the compound toward reactions expected for aromatic structures. Pyridine resists addition reactions, and resembles a benzene derivative substituted with strongly electron-withdrawing groups in both electrophilic and nucleophilic substitution reactions. As a result, extreme conditions are required for halogenation, nitration, or sulfonation reactions (electrophilic substitutions), whereas the compound is extremely reactive toward nucleophiles. Electrophilic substitution of aromatic rings generally involves the formation of carbocation intermediates with positive charges ortho or para to the position of attack. Attack of electrophiles at positions two or four results in a highly unfavored positive charge on the nitrogen atom. Because of electron deficiency, particularly at positions two and four, and electronegativity of the *N* atom, pyridine is especially resistant to electrophilic substitution. Usually, electrophylic substitution of pyridine occurs very slowly, and predominantly at position three, where the heteroatom exerts least influence. As one would predict, the pyridinium cation is even less reactive than the free base toward electrophilic substitution.

No hydroxylation of pyridine took place[64] in the Udenfriend model hydroxylation system[65] which apparently employs an electrophilic attack.[66,67] Similar results were obtained when electron withdrawing groups were included at positions which would increase electron deficiency of the ring (for example, 4-pyridinecarboxylic acid). These data suggest that pyridine should not undergo hydroxylation in biological systems via ionic mechanisms. Hydroxylation via free radical attack remains likely.

The presence of a hydroxyl group on the pyridine ring renders the ring much more susceptible to attack by electrophiles, probably due to sharing of electrons by the *O* atom. The contribution of the oxygen atom to the electron density of the ring is demonstrated by the predominance of the ketone tautomer of 2- or 4-hydroxypyridine (2- or 4-pyridone) in aqueous solutions (Figure 4). Electron density is highest ortho and para to the hydroxyl (ketone) group, therefore electrophylic attack seems more likely at these positions. In the Udenfriend system,[64] 2-hydroxypyridine (2-pyridone) produced a mixture of 2,3- and 2,5-diols. Hydroxylation of 3-hydroxypyridine occurrs at positions two, four, or five as expected. Enzymatic hydroxylation

FIGURE 4. Tautomeric ketone forms for (a) 2- and (b) 4-hydroxypyridines (pyridones).

would be expected to produce similar products. We shall see that microbial oxidation of 2-pyridone or 3-hydroxypyridine most often results in formation of a 2,5-diol. It follows that subjecting 4-pyridone to the Udenfriend system should produce a 3,4-diol (or pyridine-*N*-oxide). This was precisely what was observed.[64]

Electron withdrawing substituents, such as carboxyls, emphasize electron deficiency of the ring, and should deactivate pyridine toward electrophilic substitution, while activating it toward neucleophilic attack. We shall see that microorganisms introduce hydroxyls into pyridinecarboxylic acids via nucleophilic attack by OH- derived from water. Similar comparisons can be made of the effects of other substituents to the ring. For example, halogenated pyridines should resist electrophilic attack, whereas methylpyridines should be quite susceptible. Whereas no comparable data are available for the behavior of methyl- or halo-pyridines in the Udenfriend system, it is seen later in this review that chloropyridines persisted in soil and culture incubations, whereas methylpyridines were degraded.

Radical reactions are possible at all positions of the pyridine ring, with position two apparently favored.[5] It seems likely that radical-producing enzymes, such as laccase and peroxidase, which attack other aromatic structures,[68] could potentially hydroxylate or polymerize pyridines, regardless of the presence of electron withdrawing groups. Hydroxylation, via cometabolism for example, should activate pyridines toward electrophilic attack and thus facilitate further biodegradation. Such a reaction scheme was precedented by oxidation of pyridine to form 3-hydroxypyridine in a *Cannabis sativa* homogenate,[69] presumably as the result of peroxidase activity. We are not aware of any data on the catalytic activity of laccase with pyridine.

Because of the electron deficiency of the pyridine ring, the compound has been considered more resistant to oxidative processes than its homocyclic analogs. Pyridines were found to be refractory in Kjeldahl analyses, and required special catalysts to facilitate decomposition.[70] The stability of pyridine to oxidative attack has been demonstrated by its use as a solvent system for chromic acid oxidations of other organic compounds.[6] Pyridine was, however, susceptible to oxidation to carbon dioxide and ammonia by sodium hypochlorite[71] or potassium permanganate.[5] Similarly, the pyridine ring was cleaved in the presence of 2,4-dinitrochlorobenzene in cold alkaline solutions.[6]

As one would expect, pyridine is susceptible to reduction, yielding piperidine and intermediate hydrogenated products in the presence of hydrogen and a platinum catalyst. The pyridinium cation is more resistant to reduction than the free base. The importance of redox reactions of the pyridine ring is demonstrated by the ubiquitous occurrence of nicotinamide adenine dinucleotide as a carrier of reducing equivalents in biological systems. It will be seen next that reductive mechanisms have been proposed for pyridine degradation by both aerobic and anaerobic microorganisms.

Other important chemical properties of pyridine include miscibility with water and a broad range of organic solvents, hygroscopic nature, relatively high resonance energy (23 kcal/mol), and tendency to coordinate with metals in aqueous solution, which is discussed later.

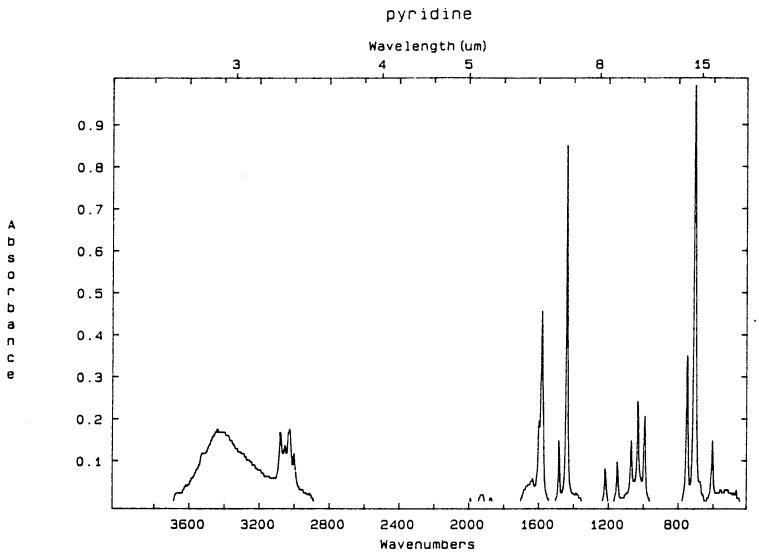

FIGURE 5. Fourier transform infrared spectrum of pyridine. (Courtesy Mattson Instruments.)

A. ANALYSIS OF PYRIDINES

Many of the properties of pyridines mentioned previously influence procedures which can be used for analyses. This is particularly true with respect to purification and concentration procedures. For example, the rather high solubility and low octanol/water partition coefficient make it difficult to extract pyridine from water into organic solvents. This characteristic also poses problems with breakthrough of pyridine in solid phase extraction techniques. The pH-dependent ability of many pyridine derivatives to form pyridinium cations allows the analyst to extract the compound either by cation exchange at low pH or partition onto nonpolar solid supports at neutral to alkaline pH. The inherent volatility of pyridines becomes an advantage in distillation of the compound from water samples, whereas it results in poor recovery when excess vacuum is applied in solid phase extraction techniques. The aromatic character of pyridines makes them strong absorbers in the UV region, and the presence of the heteroatom allows the compound to be detected by thermionic specific detectors in gas chromatography. Other unique characteristics of pyridines which pose problems or advantages in analyses are described in the paragraphs which follow.

Advantages and disadvantages of the most common procedures for concentration and purification of organic compounds have been reviewed by Jolley.[72] The most common procedures used in preparation of environmental samples for analysis of pyridines are liquid/liquid extraction in the presence of base,[73] solid phase extraction with hydrophobic[31] or cation exchange[74-76] stationary phases, and distillation in the presence of base.[77,78] Highest recoveries (90 to 100%) have been reported for distillation procedures.[77,78] Adsorption to nonpolar supports has generally produced better recovery and higher sample capacity than cation exchange procedures, whereas cation exchange procedures have been much more selective for *N*-heterocyclics in complex environmental samples.[76] Selectivity of cation exchange procedures was much more dependent upon sample pH than extraction with hydrophobic phases.[76] There was some evidence to support adsorption of the neutral species to cation exchange resins via hydrophobic interactions.

Various spectroscopic techniques have been used for detection and quantification of pyridine and related compounds. Pyridines can be detected directly by UV spectrophotometry, or by various colorimetric[79-81] and fluorimetric[82,83] procedures. Pyridines have characteristic infrared (Figure 5), nuclear magnetic resonance, and mass spectra, which have been used for qualitative

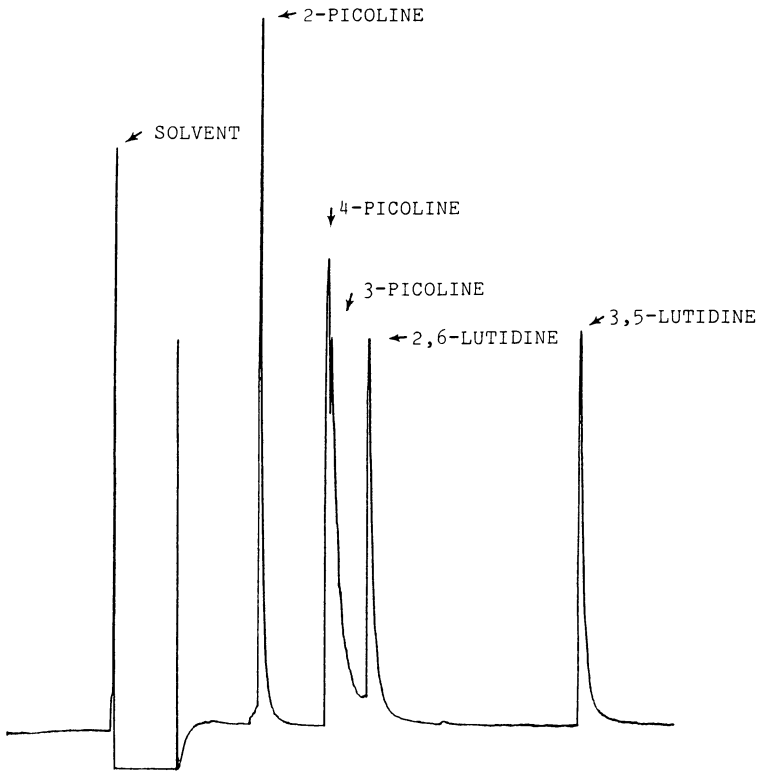

FIGURE 6. Typical capillary gas chromatogram for pyridine derivatives. Note poor separation of 3- and 4-methylpyridines.

analyses.[5] Hydroxylation results in a rather large shift of the UV absorption spectrum toward longer wavelengths, which has been used extensively in identifying metabolites of pyridine derivatives.[64,84,85]

Many chromatographic separation techniques have been applied to the determination of pyridines. Pyridines have been separated by chromatography (tlc) with silica gel[86] and aluminum oxide[87] plates. Spots on tlc plates have been visualized by reacting pyridines with cyanogen bromide and aniline or a benzidine-copper sulfate spray to form colored compounds.[6] Pyridines and related heterocyclic compounds have been detected by high pressure liquid chromatography with UV detection,[31,76] or by gas liquid chromatography with flame ionization, thermionic specific, or thermal conductivity detectors,[38,88-92] and by gas chromatography (gc) with mass specific[78,93] or vapor phase infrared[73] detectors. Some pyridine based pesticides have required derivatization for analysis by gc.[89,91] Counter-current chromatography has also been applied to determination of some heterocyclic compounds.[94]

Pyridines often exhibit peak-tailing in gas or liquid chromatography, which can be alleviated for the most part by the use of basic stationary phases,[74,77,93,95] or incorporation of surface active substances to the stationary phase.[93] Deactivation of the stationary phase appears to improve resolution.[93] As expected, capillary columns have generally provided highest resolution for pyridines, but have exhibited low sample capacities, precluding the use of relatively insensitive detection systems. Packed columns have offered much higher sample capacity than capillaries, although resolution obtained with packed columns has generally been inadequate for complex environmental samples. Therefore, a megabore column (0.75 mm I.D.) with a porous polymer stationary phase was used for gc separation of pyridines prior to vapor phase infrared spectrometry.[73] A typical capillary gas chromatogram for pyridines is shown in Figure 6.

Other technologies have shown promise for analysis of pyridines. Pyridines may be analyzed by MS/MS without chromatographic separation.[96] It appears possible to adapt colorimetric or

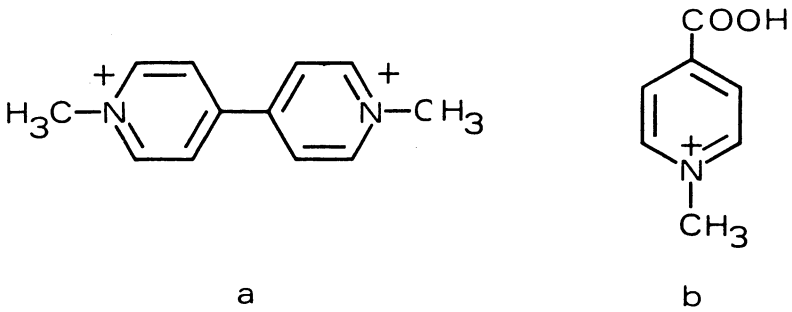

FIGURE 7. Paraquat (a), and its photolytic product *N*-methylisonicotinic acid (b).

fluorimetric procedures described previously to post column reaction methods for HPLC to increase specificity or lower detection limits. The pesticides paraquat (1,1′-dimethyl-4-4′-bipyridylium ion) and diquat (6,7-dihydropyrido{1,2-a: 2′,1′-c} pyrazinedium ion) have been determined by liquid membrane ion-selective electrodes,[97] and by enzyme-linked immunoassays.[98-100]

Nitrogen contained in the pyridine ring has been determined by sulfuric acid digestion with an appropriate catalyst[70] followed by steam distillation. Pyridine and alkyl pyridines interfered with analysis of inorganic *N* forms in steam distillation procedures, unless titrimetric determination was replaced by colorimetric or specific ion electrode detection.[101] Both Nesslerization[102] and the indophenol blue[103] procedures for ammonium were insensitive to the presence of pyridine.

V. ABIOTIC FACTORS IN THE ENVIRONMENTAL FATE OF PYRIDINES

A. PHOTOCHEMICAL TRANSFORMATIONS

Chemical transformations induced by electromagnetic radiation have long been recognized as a potentially important mechanism in the fate of organic compounds in the environment.[104-106] In the field, photodecomposition has been observed primarily in compounds which absorb radiation with wavelengths longer than 285 nm since most short wave solar energy is absorbed by ozone in the atmosphere.[106]

In aqueous samples, pyridine reacted with water when irradiated (254 nm) in neutral to alkaline environments.[107] Mathias and Hiecklen,[108] reported formation of hydrocarbons, pyridine isomers, polymers, and hydrogen when pyridine was irradiated with short wavelength UV (wavelengths between 213 and 229 nm). However, pyridine resisted photochemical degradation in the vapor phase when irradiated with wavelengths longer than 250 nm.[109,110] Therefore, direct photolysis of the unsubstituted pyridine ring by sunlight seems unlikely. Photolysis may be enhanced by the presence of photosensitizers in natural waters or other mixed media.

Substitution of the pyridine ring markedly alters absorption maxima. For example, aminopyridines and hydroxypyridines generally demonstrate at least one absorption maximum above 280 nm,[63] and may be susceptible to photochemical degradation in the environment. It therefore was not surprizing that some hydroxylated metabolites of pyridine based pesticides, such as 6-hydroxypicolinate derived from nitrapyrin, were susceptible to photochemical transformation.[111] Other pyridine derivatives, particularly pesticides, have demonstrated photosensitivity as well. Both paraquat and diquat were photolytically cleaved to yield the simple pyridine derivatives, *N*-methylisonicotinate (Figure 7) and picolinamide (Figure 8), respectively.[112-114] Adsorption of paraquat to soil or plant surfaces shifted the UV absorption maximum from 257

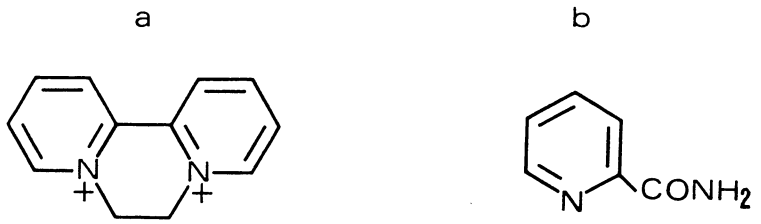

FIGURE 8. Diquat (a), and its photolytic product, picolinamide (b).

to 275, and therefore increased susceptibility of the compound to photodecomposition.[112] Photodecomposition of diquat was independent of surface adsorption.[113] Another pyridine based herbicide, picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) was also photolytically degraded.[115-117] There has been evidence to support photochemical decomposition of the soil-applied herbicide imazaquin {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imiddazol-2-yl}-3-quinolinecarboxylic acid}[118] and the pyridine analog, imazapyr (2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid) which was decomposed in aqueous media in simulated sunlight at pH 5 to 9.[119] Haloxyfop {2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid} underwent photolysis in aqueous solution when exposed to UV radiation (300 to 400 nm) from a synthetic source.[120] Rates of photolysis of the compound were increased up to five- to sevenfold in the presence of 0.15% (v/v) polyoxyethylene sorbitan monolaurate or when irradiated in an organic solvent (paraffin oil).

B. VOLATILIZATION OF PYRIDINES

Pyridine and most alkylpyridines are volatile, with vapor pressures of approximately 0.15 MPa at 25°C.[63] Chlorinated pyridines such as pesticides exhibit vapor pressures of <0.01 to 5 MPa at 25°C.[119] Volatility of pyridines associated with energy related wastes has resulted in detection of these compounds in air samples.[39] Volatilization accounted for up to 57% of the loss from solution of methyl- and chloropyridines in soil suspensions,[101] although little volatilization loss was reported in an experiment with whole soil.[121] Volatility was also an important factor in the fate of nitrapyrin (2-chloro-6-(trichloromethyl)pyridine), an inhibitor of ammonium-oxidizing bacteria, which was lost from soil primarily due to volatilization.[122]

C. TRANSPORT OF PYRIDINES IN THE ENVIRONMENT

Movement of pyridines through the environment is expected, due to the high water solubility of the compounds. Transport of pyridines in the environment is well documented. Pyridines and related heterocycles are mobile in soils,[36] and have been observed in groundwaters proximate to underground coal gasification sites.[2,123] Pyridines have been detected in wastes from energy related processes,[38,124-127] which were intended for land disposal. The pyridine based herbicide picloram is mobile in soils,[128-131] and has been detected in surface and groundwaters as a result of agricultural runoff,[40,132-135] although concentrations observed have seldom exceeded tolerance thresholds. Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) a common insecticide and a metabolite (3,5,6-trichloro-2-pyridinol), have been detected in surface waters as a result of agricultural and public health usage.[136-138]

Recently developed pyridine-based herbicides appear generally less mobile in the environment than pyridine and alkylpyridines derived from synfuel processes or picloram and chlorpyrifos applied to agricultural fields and rangelands. Imazapyr remained within 7.5 cm of the treated area after 1 year of incubation in the field.[139,140] Little appreciable lateral transport was noted. Downward transport (5-25 cm) of fluazifop-butyl {butyl 2-[4-(5-trifluoromethyl-2-pyridoxy)phenoxy]propionic acid} and haloxyfop-ethoxyethyl {2-ethoxyethyl-2-[4-(3-chloro-5-trifluoromethyl-2-pyridyloxy)phenoxy]propionic acid} was observed within three months of

application to field soils.[141] Triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid and an ethylene glycol butyl ester were relatively immobile through a loam soil (less than 10 cm downward movement in 54 d) but passed rapidly through sand[142] when 2.5 cm aliquots of water were leached through the soil on an alternate day cycle.

D. COMPLEXATION WITH INORGANIC AND ORGANIC SPECIES IN THE ENVIRONMENT

Pyridine and its derivatives often form complexes with metals in aqueous solutions. Pyridine-2-carboxylic acid (picolinic acid) and derivatives coordinate strongly with metals.[143] Picloram formed weak complexes with Fe(III) and Cu(II)[62] and strong, insoluble complexes with Fe(II) and Ni(II).[144] Formation of such complexes through the introduction of Fe(II) could serve to remediate picloram in spills, or may function in nature to remove the compound from solution upon arrival in groundwater. Hydroxypyridines and pyridones also coordinate with metals. Colored complexes formed by reaction of pyridinediols and $FeCl_2$ have been used with chromatographic mobility for identification of the compounds in culture media.[64] Complexes between other pyridines and metals probably occur in the environment, and may contribute to the environmental fate of these compounds.

Pyridine and related compounds are adsorbed via ionic mechanisms to mineral surfaces in soil.[145] Sorption of binary mixtures of similar heterocyclic compounds (e.g., pyridine and quinoline) to subsurface sediments was competitive at low pH when the compounds were at least partially ionized, whereas sorption was noncompetitive when only the neutral species was present.[145] Ionic interactions of pyridines with mineral surfaces seemed most likely when the solution pH was near the pKa of the compound. At higher pH values, association of the neutral species with organic constituents of the soil matrix seemed likely. Similarly, adsorption of pyridines to cation exchange resins was strongly pH dependent.[76] In this experiment, pyridine appeared not only to undergo ionic interaction with the exchanger, but also to interact with nonpolar surfaces in the exchange resin. Interactions between pyridines and nonpolar organic phases have been observed even for permanently charged heterocyclic species.[146]

Fluridone [1-methyl-3-phenyl-5-(3-trifluromethyl)phenyl]-4-(1H)-pyridinone] was adsorbed to organic and mineral constituents of 18 soils studied.[147] Adsorption increased as pH was decreased from 6.4 to 3.5. The compound was much more readily desorbed into solution at the higher pH level. Data suggested that ionic interactions of the herbicide with the exchange site was much more important in acidic solution.

Pyridines migrated through alkaline soils (pH = 8.6) associated with oil shales of Wyoming in exactly the same order as they eluted from a reverse phase (C18) HPLC column.[36] The results suggested that movement of the compounds through the soil under investigation was controlled by hydrophobic interactions between the pyridines and soil organic matter. Wastewaters produced in processing oil shales of the eastern U.S. also contained pyridine derivatives,[148] but were generally acidic (pH = 4.3 to 5.1) rather than alkaline.[149] Should elution of pyridines through these soils differ significantly from soils of the western U.S.?

VI. BIOTIC FACTORS IN THE ENVIRONMENTAL FATE OF PYRIDINES

A. APPARENT BIODEGRADABILITY OF PYRIDINES

Although pyridine is readily degraded by microorganisms, biodegradability of pyridine derivatives appears to be affected rather dramatically by the nature and position of ring substituents.[101,121,150] Pyridine and pyridinecarboxylic acids appeared to be most susceptible to aerobic degradation, usually disappearing in less than 7 d in soil suspensions, whereas chloro- and aminopyridines were most persistent, usually requiring more than 30 d for complete disappearance.[101] Increasing the number of halogen substituents increased persistence of the

pyridine ring. Among pyridinecarboxylic acids, 2,4-pyridinedicarboxylic acid was unusually persistent, requiring 24 d for disappearance. Monohydroxypyridines and pyridinones were slightly more persistent than pyridinecarboxylic acids (requiring 7 to 24 d for disappearance). Most methylpyridines were degraded within 24 d, although 3-methylpyridine and 2,6-dimethylpyridne were present through 30 d of incubation. Essentially identical results were observed when experiments were performed with whole soil.[121] Naik et al.[150] performed a similar experiment, and achieved similar results, although there were some unusual findings, such as little or no difference between aerobic and anaerobic treatments and the apparent persistence of pyridine and monohydroxypyridines. These major discrepancies could have been attributed to adsorption phenomena and oxygen limitation in the static cultures used.[101]

B. AEROBIC METABOLISM OF PYRIDINES BY MICROORGANISMS

Of great interest in the study of the metabolism of aromatic rings are the various mechanisms involved in ring fission. In aerobic environments, homocyclic rings are usually activated by hydroxylation, usually to form a diol, which undergoes fission when further oxidized. Generally, mono- and/or dioxygenase activities are involved, therefore oxygen atoms in the resulting hydroxyls are derived from O_2. Examination of the literature on the metabolism of pyridine rings reveals three general mechanisms for activating the rings prior to fission. In the case of pyridinecarboxylic acids, the ring is usually hydroxylated via nucleophilic attack by –OH derived from water. In the case of hydroxypyridines, a second hydroxyl is generally introduced by monooxygenase attack or nucleophilic addition at electron deficient sites. The most novel mechanism, and probably least understood, is the fission of pyridine and some alkylpyridines by a mechanism which apparently does not involve hydroxylated intermediates. The proposed mechanism usually includes an initial reduction step to form a dihydropyridine, which is then cleaved to release saturated aliphatic intermediates. What follows is a detailed description of what is known about the mechanisms for degradation of various pyridine derivatives. Discussion is divided into separate topics based upon the nature and position of ring substituents. In most cases, a single type of reaction mechanism has been observed for a particular compound, however, in some cases, several different pathways have been proposed.

1. Niacin and Related Compounds

Bacterial metabolism of nicotinic acid and nicotinamide have been studied extensively. Results of these investigations have formed a basis for conjecture about the metabolic fate of many other pyridines. Numerous authors have reported growth of bacteria on nicotinic acid under either aerobic[84,151,152,154-161] or anaerobic[162-164] conditions. Metabolic studies confirmed 6-hydroxynicotinate as the first intermediate in aerobic catalysis of nicotinic acid.[84,164-166] By far the most referenced work is that of Behrman and Stanier[84] in which the authors proposed a complete pathway for nicotinate metabolism in a strain of *Psuedomanas fluorescens* (later reclassified as *Pseudomonas putida*).[167] This organism produced 6-hydroxynicotinate which was oxidatively decarboxylated to 2,5-dihydroxypyridine (5-hydroxy-2-pyridone). The reaction was originally thought to be the result of two successive monooxygenase attacks, though the mechanism was not clearly demonstrated. Using ^{18}O-labeled water and O_2, Hunt et al.[159] showed that the first hydroxyl was derived from water rather than O_2 (Figure 9). The enzymes involved in these reactions appeared to be associated with cytochromes in *Pseudomonas putida,* but were found in the soluble fraction in *Bacillus.*[160] Subsequent oxidation of the diol by 5-hydroxy-2-pyridone oxygenase probably produced *N*-formylmaleamic acid, which was then hydrolyzed nonenzymatically[168] to formate and maleamate, which has become a pseudonym for the pathway. Using ^{18}O, Gauthier and Rittenberg[169] demonstrated a dioxygenase mechanism for the 5-hydroxy-2-pyridone oxygenase. Some disagreement existed as to whether the first product of ring fission was *N*-formaylmaleamate or *N*-formylfumarate.[168-170] Authorities did, however, agree on the identity of the hydrolysis product of *N*-formylmaleamate (or *N*-formylfumarate) as

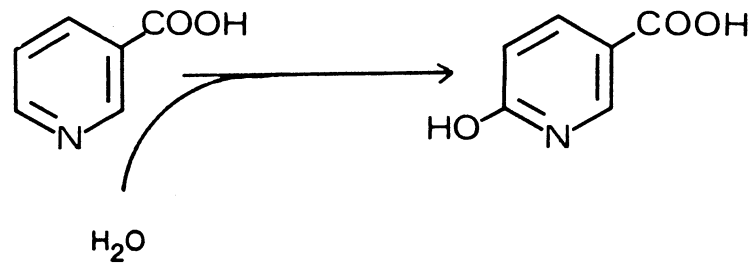

FIGURE 9. Hydroxylation of nicotinic acid to produce 6-hydroxynicotinate. This reaction occurs in both aerobic and anaerobic environments.

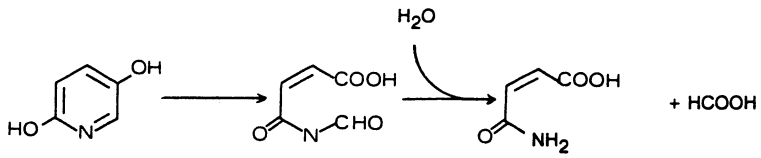

FIGURE 10. Fission of 5-hydroxy-2-pyridone (probably via dioxygenase attack) to produce *N*-formylmaleamic acid, and subsequent hydrolysis to release maleamate and *formate.

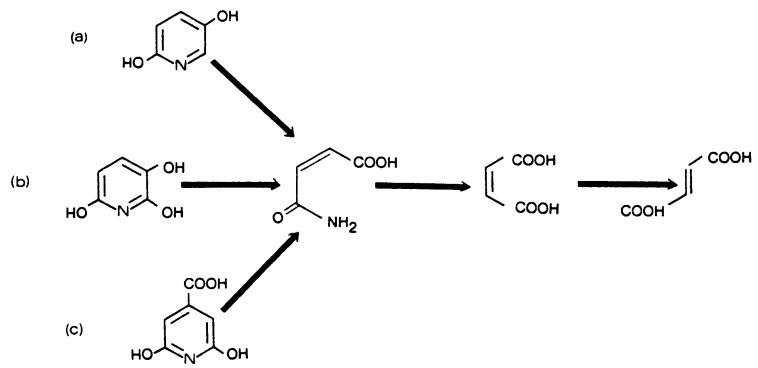

FIGURE 11. Convergence of three metabolites, (a) 5-hydroxy-2-pyridone, (b) 2,3,6-trihydroxypyridine, and (c) 2,6-dihydroxyisonicotinic acid, into the maleamate pathway.

maleamate (Figure 10). The remainder of the pathway involved preparing maleamate for entry into the citric acid cycle. Maleamate was deaminated to produce maleic acid, which could be isomerized to produce fumarate.

Ensign and Rittenberg[160] proposed a modification of the original maleamate pathway to include 2, 3, 6-trihydroxypyridine as a central intermediate which would allow convergence of 2,5-dihydroxypyridine formed by *Pseudomonas* and 2,6-dihydroxypyridine formed by *Bacillus* to enter a common maleamate pathway (Figure 11). The proposed triol would also provide a logical subtrate for production of the characteristic azaquninone pigment via nonenzymatic polymerization. Subsequent investigations resulted in purification of nicotinate hydroxylase and 6-hydroxynicotinate hydroxylase from the soluble cell fraction.[171] The enzymes were large (molecular weight = 400,000-450,000) and contained both flavin and iron. The enzymes were shown to be coordinately induced by 6-hydroxynicotinate.[172]

Metabolism of nicotinate by the maleamate pathway has been reported for other organisms, although few details of the biochemistry were provided.[158,161,173] *Rhizobium* sp. strain ORS571

was able to use N_2 and nicotinic acid synergistically as N sources.[161] It appeared that 6-hydroxynicotinate hydroxylase and 5-hydroxy-2-pyridone oxidase activities in this organism scavenged 2 mol of intracellular O_2 per mol of 6-hydroxynicotinate oxidized, and therefore provided an anaerobic environment suitable for activity of nitrogenase. Nicotinate metabolism by *Sarcina* sp.,[158] and a Gram-negative coccus[173] also involved 6-hydroxynicotinate, 5-hydroxy-2-pyridone, and maleamate.

Rhodococcus rhodochrous, a benzonitrile-catabolizing organism isolated by enrichment from soil, was able to convert 3-cyanopyridine to nicotinic acid via nitrilase activity.[174] This reaction has potential application in the synthesis of nicotinate from 3-cyanopyridine, which is normally achieved by refluxing with $Ba(OH)_2$. The nitrilase has a broad substrate specificity range and may be applicable for removal of labile nitrile groups from a variety of compounds. Similar reactions may be exploited for the detoxification of cyanide in cyanogenic foods such as cassava.

Numerous articles have been written on bacterial metabolism of nicotinamide.[175-184] Most of the information available has been provided by Snell and co-workers. Proposed reaction sequences for nicotinamide degradation included oxidation of methanol groups at positions three and four of the pyridine ring to yield corresponding acids. Ring cleavage occurred between carbons at positions two and three of the ring via dioxygenase attack. It is worthwhile to note that initial oxygenase attack on nicotinamide involved a concomitant reductive step[183] (Sparrow et al.). Several subsequent papers dealing with metabolism of pyridines[1,185-191] evoked the concept of an initial reductive step, although in only one other case was reduction confirmed experimentally.[191]

2. Picolinate, Isonicotinate, and Dipicolinate

Shukla and Kaul[192] described degradation of 2-pyridinecarboxylic acid by a *Bacillus* sp., which produced 6-hydroxypicolinic acid, 3,6-dihydroxypicolinic acid, and 2,5-dihydroxypyridine as intermediates. The authors proposed a variation of the maleamate pathway for the remaining steps in the catabolic pathway. Metabolism of picolinate, via the maleamate pathway has been described by several authors.[173,193,194] Orpin et al.,[195] described degradation of picolinamide, a photolytic product of diquat, in which a Gram-negative rod metabolized the substrate via the maleamate pathway after initial deamination and hydroxylation at position six. The hydroxylation step apparently involved incorporation of oxygen derived from water.

Isonicotinic acid (4-pyridinecarboxylic acid) was degraded by a Gram-negative rod via a pathway involving a 2,6-diol derivative of isonicotinate (citrazinic acid), and the production of a presumably azaquinone pigment.[160] No further information was available on the catabolic pathway. Wright and Cain[191] proposed a reaction mechanism for dissimilation of *N*-methylisonicotinate, a photolytic product of paraquat. In their study, *Achromobacter* D (probably a species of *Acinetobacter*) cleaved the ring between carbons at positions two and three to produce a dialdehyde. The pathway did not appear to require hydroxylated intermediates, and apparently involved an initial reductive step which required NADH. Orpin et al.,[185] described degradation of *N*-methylisonicotinic acid by a pathway invoving a hydroxylation step which did not require molecular oxygen. Hydroxylation was followed by demethylation, and subsequent metabolism of the resulting diol via the maleamate pathway (Figure 11). No initial reductive step was proposed. *Bacillus brevis* degraded isonicotinate, 2-hydroxyisonicotinate, and 2-pyridone.[196] Both 2-pyridone and 2-hydroxyisonicotinate were apparently converted to 5-hydroxy-2-pyridone and further degraded by the maleamate pathway. Data suggested that isonicotinate was reduced[197] prior to ring fission as proposed previously by Wright and Cain.[191]

Bacillus sp. have been shown to produce 2,6-pyridinedicarboxylic acid (2,6-dipicolinate) during sporulation. Because of the natural occurrence of dipicolinate, it is not surprizing that the compound can serve as a carbon and nitrogen source for microorganisms.[198] *Achromobacter* (probably *Acinetobacter*) sp. converted 2,6-dipicolinate to a 3-hydroxy derivative and released

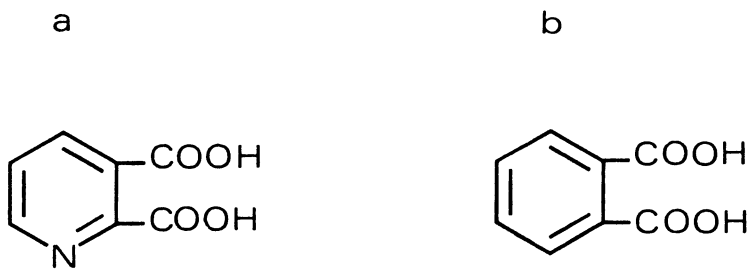

FIGURE 12. Dipicolinate (2,3-pyridinedicarboxylic acid) (a) and its homocyclic analog, o-phthalate (b).

2-oxoglutarate after ring fission.[199] Taylor[200] and Taylor and Amador[201] reported stimulation of oxygen uptake by 2,3-, 2,5-, or 2,6-dipicolinic acid in marine bacteria isolated on appropriate phthalate analogs (ortho-, meta-, and para-phthalates) of the picolinates. Evidence of chemical transformation of dipicolinate was provided, although metabolites were not isolated. A mixed culture isolated on 2,3-dipicolinate (Figure 12a) did not oxidize the structural analog, o-phthalate (Figure 12b).[202] The authors concluded that even though the picolinate degraders were not able to oxidize phthalates, the nonspecific hydroxylase system of the phthalate degraders was apparently able to oxidize pyridines.

3. Hydroxypyridines (Pyridones)

A significant amount of information is available on the biochemical mechanisms for biodegradation of monohydroxypyridines. Two general degradative pathways have been recognized. Degradation of either 2-pyridone or 3-hydroxypyridine was thought to proceed via the maleamate pathway,[64] whereas 4-pyridone was converted to a 3,4-diol which produced pyruvate, formate, and ammonium upon ring fission.[85,203]

Three isolates, identified as *Achromobacter* sp. (probably *Acinetobacter* sp.) were found to produce 5-hydroxy-2-pyridone from either 2-pyridone or 3- hydroxypyridine.[64] The results from respiration experiments suggested that the oxygen atom for the hydroxylation reaction was derived from water for either of the two mono-hydroxypyridines. Subsequent investigations with two of the isolates resulted in the elucidation of the remainder of the degradation pathways.[204] Fission of the 2,5-diol by either isolate produced maleamate and formate, which suggested the presence of a maleamate pathway as described previously by Behrman and Stanier.[84] *Arthrobacter crystallopoietes,* an extensively studied organism, was originally isolated by enrichment with 2-pyridone.[205] In a series of manuscripts dealing with this particular organism, and two related arthrobacters (*A. pyridinolis* and *A. globiformis*) it was proposed that the organisms possessed a 2,6-dihydroxypyridine oxygenase which was responsible for ring cleavage.[206,207] Relatively little detail of the metabolic pathways was presented, thus, most of the steps involved remained uncertain. *A. crystallopoietes* appeared to carry a 63 Mdal plasmid which encoded the 2,5-dihydroxypyridine monooxygenase involved in ring fission and formation of a blue pigment.[206,208] The organism was also able to degrade pyridine, although loss of the plasmid did not affect this capability.[209,210] We know of no other information regarding the genetics of pyridine degradation.

Houghton and Cain[64] isolated an *Agrobacterium* sp. which was able to degrade 4-pyridone, via a 3,4-diol intermediate (Figure 13). Formation of this intermediate required 1 mol of O_2/mol of 4-pyridone oxidized, suggesting involvement of a mono-oxygenase. In a subsequent paper,[203] isolation of a pyridine-3,4-diol oxygenase was described. The enzyme had a molecular weight of approximately 330,000, and was labile *in vitro* ($t_{1/2}$= 4 h), but could be stored successfully in an anerobic environment. Because the enzyme required O_2, but not other cofactors such as NADH, it was classified as a dioxygenase, and was compared to extradiol (meta) oxygenases associated with cleavage with homocyclic rings.[85,203] The product of the dioxygenase attack was

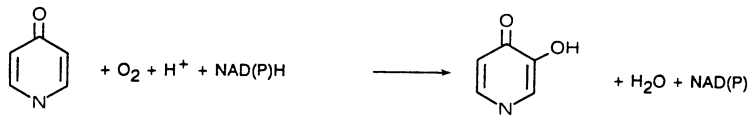

FIGURE 13. Oxygenase attack on 4-pyridone to produce a 3,4-diol.

3(*N*-formyl)-foriminopyruvate, which was hydrolyzed to produce formate and 3-formiminopyruvate. Removal of ammonia by hydrolysis produced 3-formylpyruvate, which was hydrolyzed to pyruvate and formate.[85]

It is interesting to note the incorporation of oxygen derived from water to form 2,5-dihydroxypyridne from either 2-, or 3-hydroxypyridine,[64] whereas molecular oxygen was directly incorporated to produce the 3,4-diol from 4-hydroxypyridine.[203] These results were somewhat surprizing due to the predicted susceptibility of all three compounds to monooxygenase attack. As mentioned previously, other organisms have been reported to use oxygen derived from water to hydroxylate pyridine derivatives, particularly pyridinecarboxylic acids.[159,211,212] Such a mechanism was not surprizing for the carboxylic acids, because the ring should be deactivated toward electrophilic attack due to the electron-withdrawing nature of the carboxyl. Thus, the probability of nucleophilic attack by –OH to introduce hydroxyl groups into pyridinecarboxylic acids was precedented by the chemistry of the compounds. Perhaps the hydroxylases which formed the 2,5-diol were recruited from the maleamate pathway, which may have evolved for degradation of carboxylic acids such as nicotinate. Such possibilities were not examined in the original manuscripts.

4. Pyridine

A number of laboratories have reported isolates capable of growth on unsubstituted pyridine, and several attempts have been made to propose pathways for metabolism by microorganisms. All of these attempts thus far have been hindered by difficulty in obtaining aromatic metabolites, and the inability to produce cell-free extracts with activity on pyridines. Houghton and Cain[64] reported isolation of *Nocardia* Z1 which grew at the expense of pyridine, but was not able to grow on or oxidize hydroxypyridines, with the exception of 3-hydroxypyridine. The organism used 3-hydroxypyridine very slowly (1/30 the rate of pyridine oxidation), and 3-hydroxypyridine was therefore discounted as an intermediate in pyridine metabolism. It should be noted that it was not known that the organism was permeable to 3-hydroxypyridine (a polar aromatic sp.). In order to remove interference from permeability barriers, the authors examined cell-free extracts for activity against 3-hydroxypyridine, however the data were inconclusive since extracts were not only unable to oxidize 3-hydroxypyridine, but were also unable to oxidize the parent compound (pyridine) as well. Subsequent experiments with this organism, and a *Bacillus* sp. resulted in a conclusion that hydroxypyridines were not involved in pyridine metabolism (although permeability of cells to hydroxypyridines was never proven), and that pyridine was initially reduced prior to ring cleavage.[190] Additional support for ring reduction was found in the appearance of ring fission products as fully reduced aliphatic acids or semialdehydes rather than alkenes.

The experiments published by Watson and Cain[190] and contemporaneously by Shukla and Kaul[187,188] appear to be the most complete investigations of pyridine metabolism available. The two laboratories, working simultaneously (the first two detailed manuscripts were received by the journals within a 2-month period), came to almost exactly the same conclusions about pyridine metabolism. The *Nocardia* sp., studied by Watson and Cain[190] cleaved the ring between the nitrogen atom and adjacent carbon to produce ammonium and glutaric dialdehyde. Ring cleavage was thought to be initiated via a hypothetical 1,4-dihydropyridine intermediate. The authors also reported that a *Bacillus* sp. cleaved the ring between the carbons at positions two and three (via a hypothetical 1,4-dihydropyridine intermediate) releasing formamide and

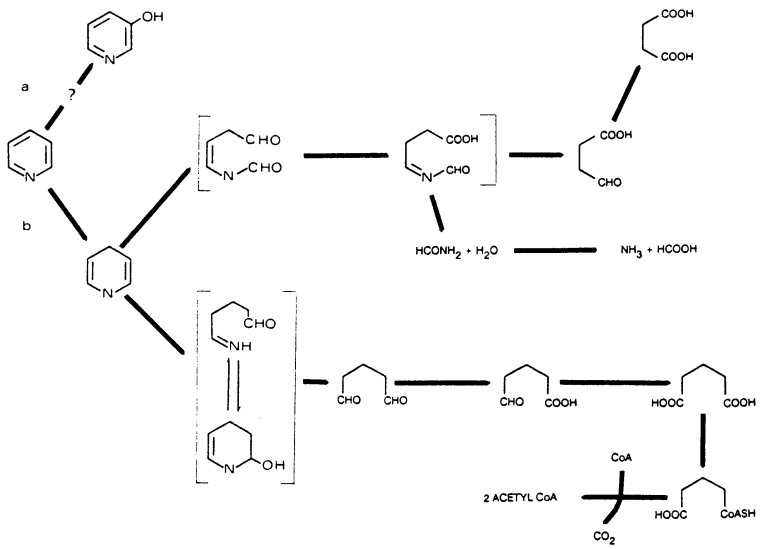

FIGURE 14. Metabolism of pyridine by microorganisms via oxidative (a) and reductive (b) mechanisms.

succinate semialdehyde (Figure 14). Almost identical results were reported by Shukla and Kaul[187,188] in studies of pyridine metabolism by *Brevibacterium* and *Corynebacterium* sp. Both of these organisms apparently cleaved the pyridine ring in exactly the same manner as the *Bacillus* sp. reported by Cain's group. Again, succinate semialdehyde was the first metabolite observed. Shukla and Kaul[187,188] also proposed an initial reductive step. Shukla and Kaul[189] later reported similar observations for another *Nocardia* sp. isolated by enrichment on pyridine-N-oxide. Both laboratories reported inability of the organisms to degrade hydroxypyridines, and neither laboratory was able to produce cell-free extracts with catabolic activity toward pyridine or pyridine derivatives, regardless of induction status, or methods used to disrupt cells. Cleavage of pyridine between C-2 and C-3 and subsequent release of succinate semialdehyde has been reported for another *Nocardia* sp.[213] and for *Micrococcus luteus*[1]. Neither of these organisms were able to oxidize hydroxypyridines. In the case of *M. luteus*, the organism was apparently permeable to most mono- and dihydroxypyridines. Again, the authors were unable to produce functional cell-free extracts.

Korosteleva et al.[213] proposed involvement of 3-hydroxypyridine in pyridine degradation by another *Nocardia* isolate, although the organism studied was unable to oxidize 3-hydroxypyridine (Figure 14). No data were available to demonstrate permeability of the organism to hydroxypyridines. *Arthrobacter crystallopoietes*, which has been studied extensively as a 2-pyridone-degrader, was also able to degrade pyridine by an inducible system,[210] however, utilization of pyridine was not coordinately induced by growth on 2-pyridone.[210] Neither was 2-pyridone degradation induced by growth on pyridine. A 63 Mdal plasmid necessary for 2-pyridone utilization was not necessary for pyridine degradation.[214] Therefore, it appeared that the organism had either a branched pathway for degradation of pyridine rings, or possessed two distinctly different pathways.

Shukla and Kaul[189] reported metabolism of pyridine, pyridine-*N*-oxide, and 2-pyridone by a *Nocardia* species. Induction patterns for biodegradation of pyridines followed patterns analogous to those observed in *A. crystallopoietes* above. Cells grown on pyridine-*N*-oxide were cross-adapted for growth on 2-pyridone, but not pyridine. The authors suggested that metabolism of pyridine-*N*-oxide and 2-pyridone proceeded via the formation of a 2,5-diol and perhaps a 2,3,6-triol, which could enter a maleamate pathway. A separate pathway was proposed for pyridine metabolism, which was thought to involve ring reduction to facilitate fission. Like *A. crystallopoietes*, this *Nocardia* produced a blue azaquinone pigment, possibly as the result of

non-enzymatic polymerization of a 2,3,6-trihydroxypyridine. The pigment was formed during growth on 2-hydroxypyridine or pyridine-*N*-oxide but not during growth on pyridine. *Arthrobacter crystallopoietes* also failed to produce pigment in the presence of pyridine.[214]

Data available thus far suggested that pyridine was metabolized via a novel mechanism of ring fission, possibly involving an unstable reduced intermediate, and possibly bypassing hydroxylation reactions ubiquitous to metabolism of both homocyclic and heterocyclic compounds. The repeated observation that pyridine degraders (as well as organisms grown on *N*-methylisonicotinate and some alkylpyridines) did not use hydroxypyridines, and produced reduced aliphatic metabolites is difficult to overlook. Resistance of pyridine to hydroxylation is predicted by its chemistry, and precedented by its nonreactivity in a model hydroxylating system. However, it should be recalled that pyridine was hydroxylated enzymatically by plant peroxidases.[69] To date, there has been no conclusive published evidence to support a role of hydroxypyridine intermediates in the degradation of the unsubstituted pyridine ring, even though there is substantial evidence to demonstrate hydroxylation of substituted pyridines. Likewise, the theoretical 1,4-dihydropyridine intermediate has not been isolated from cultures or cell fractions supplied with pyridine. Thus, the nature of the early steps in pyridine metabolism remain obscure.

5. Alkylpyridines

Relatively little information is available on biodegradation of alkyl pyridines, which constitutes the largest class of pyridines detected as pollutants in the environment. Shukla[215] reported degradation of 2-methylpyridine by an *Arthrobacter* sp., which also appeared to reduce the ring prior to ring cleavage, and apparently produced succinate semialdehyde as an intermediate. Aromatic metabolites were not observed. This organism was not able to degrade hydroxylated analogs of 2-methylpyridine, but was able to utilize reduced substances, such as piperidine. Like the *Micrococcus* grown on pyridine as described previously, this organism produced a yellow pigment tentatively identified as riboflavin during growth on 2-methylpyridine. The authors were unable to identify a role for riboflavin overproduction during the degradation process. Additional studies were performed with 2-, and 4-methylpyridines, and with 2,4- and 2,6-dimethylpyridines.[186,215] Conversely, *Psuedomonas* sp. apparently converted 3-methylpyridine to the corresponding carboxylic acid, which then entered the maleamate pathway.[213] Analogous reactions were observed during cometabolism of 2-methylpyridine and 3-methylpyridine to produce the corresponding acids.[216]

C. FORMATION OF PIGMENTS DURING GROWTH OF MICROORGANISMS ON PYRIDINE DERIVATIVES

Pigment production has been a common feature associated with growth of microorganisms on pyridine derivatives. Ensign and Rittenberg[205] described production of a blue pigment during growth of *Arthrobacter crystallopoietes* on 2-hydroxypyridine. Pigment production was also associated with growth of *Bacillus* sp. on nicotinate[160] and growth of *Arthrobacter* sp. on nicotine.[217-219] It was postulated that these pigments were produced from the chemical oxidation of tripyridols which arose spontaneously from 2,6-dihydroxypyridines formed during degradation of nicotinate and nicotine.[160] Formation of azaquinones from tripyridols has been documented.[220-222] *Micrococcus* produced riboflavin during growth on pyridine.[214] Riboflavin was not produced directly from pyridine since radiolabel introduced as 2,6 ^{14}C-pyridine did not appear in riboflavin produced during growth of the organism. It is not known what role (if any) riboflavin served in pyridine degradation. Perhaps pyridine derepressed riboflavin biosynthesis through interactions with repressor proteins required for transcription of genes encoding riboflavin synthase or GTP cyclohydrolase, key points in the control of riboflavin biosynthesis.[223] Overproduction of riboflavin was also reported for *Arthrobacter* sp.[215] (Shukla) grown on 2-methylpyridine.

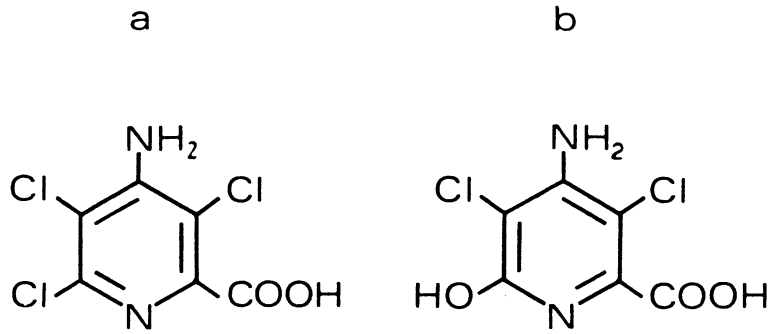

FIGURE 15. Picloram (a) and metabolite (b), formed in soil under nonsterile conditions.

D. ANAEROBIC METABOLISM OF PYRIDINES BY MICROORGANISMS

Anaerobic metabolism of heterocyclic compounds, including pyridine derivatives has been reviewed recently.[224] Very little information is available on anaerobic degradation of pyridine derivatives. The best known studies have involved fermentative growth on nicotinic acid. *Clostridium* sp. grew on nicotinate under anaerobiosis, and produced 6-hydroxynicotinate, acetate, propionate, ammonium, and CO_2.[162-164] Further investigations were not possible as the culture was lost. *Clostridium barkeri*[225,226] produced the same end products as described previously. Again, 6-hydroxynicotinate was identified as the first metabolite detected. Further investigation resulted in the isolation of an nicotinic acid hydroxylase (molecular weight =300,000), which required NADP, and produced a hydroxyl group with oxygen derived from water. The enzyme appeared to contain selenium.[227] The fate of most environmentally significant pyridines in anaerobic environments remains unknown.

E. DEGRADATION OF PYRIDINE DERIVATIVES IN NATURAL SYSTEMS

It has been difficult to extrapolate from laboratory data as to what the fate of a particular compound should be in nature, although available evidence suggested that a number of pyridine derivatives exhibit similar behavior in both soils and cultures.[101,121] Information on the fate of pyridines in the environment is for the most part lacking. Early reports showed that pyridine was rapidly mineralized in soil,[228-230] however, little is known of the biodegradation of alkylpyridines in the environment. This information gap is important, in light of the widespread occurrence of alkylpyridines in the environment.

Perhaps the most extensive database on the environmental fate of substituted pyridines comes from the pesticide literature. Pyridine-containing pesticides for which environmental fate is discussed include picloram (4-amino-3,5,6-trichloropicolinic acid), nitrapyrin (2-chloro-6-trichloromethylpyridine), chlorpyrifos (*O,O*-dietyl *O* -3,5,6-trichloro-2-pyridyl phosphorothioate), flouridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4-(1H)pyridinone}, nonflurazon [4-chloro-5-(methylamino)-2-(3-(trifluoromethyl)phenyl)-3(2H)-pyridazone], triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid], and 4-aminopyridine.

The fate of picloram in soil and natural waters has been studied by a number of investigators.[231,232,134] Meikle et al.[231] proposed that the compound was converted to the 6-hydroxy derivative in soil (Figure 15). The half-life of picloram in soil has ranged from 30 to 330 d depending upon soil conditions.[119]

Nitrapyrin, an effective inhibitor of autotrophic ammonium oxidizing bacteria, was rapidly dechlorinated to produce 6-chloropicolinic acid (Figure 16) in soil.[233] The mechanism for dissimilation of the metabolite was not described. Significant volatilization losses were also reported.

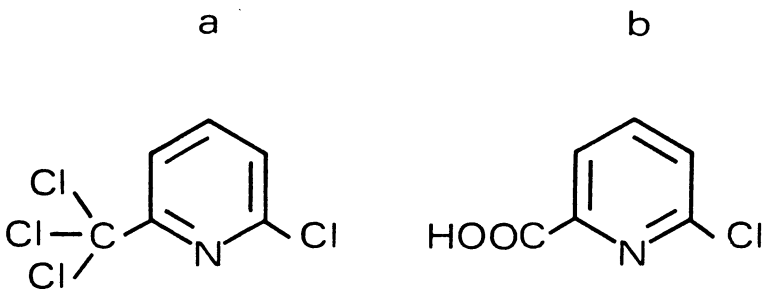

FIGURE 16. Nitrapyrin (a) and metabolite (b), formed in nonsterile soil.

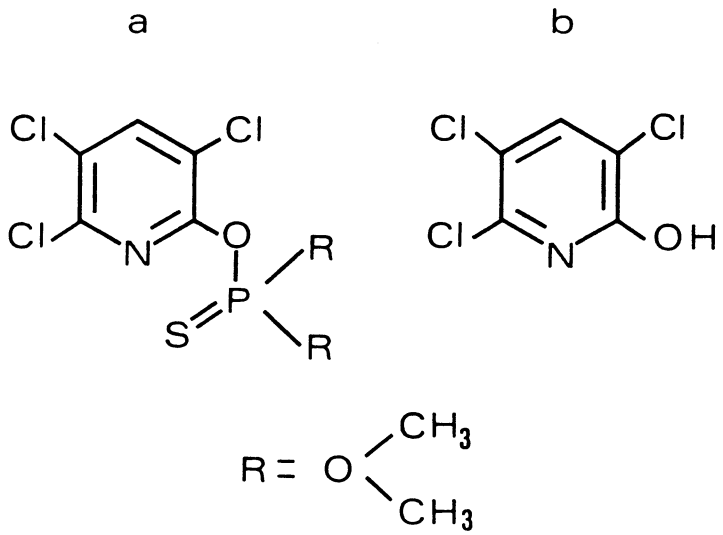

FIGURE 17. Chlorpyrifos (a) and metabolite (b) formed in soils and natural waters.

Chlorpyrifos, a pyridine based insecticide, was degraded more rapidly in sand than in muck, suggesting an negative effect of organic matter on degradation rate.[234] The half-life of the compound in an Italian soil was approximately 1 month.[138] During the initial degradation period, the compound was converted almost quantitatively to a pyridinol metabolite (Figure 17), which persisted for several months. The data supported the implicit assumption that ring fission rather than removal of the labile phosphorothioate group controlled the rate of biodegradation.

Flouridone persisted for 250 to >385 d in soils.[235,236] Dissipation of the compound from soil appeared to be biologically mediated,[236] and was enhanced by previous treatment of the soil with the compound.[235,237] Approximately 30% of the compound remained unaltered in submerged soil after 1 year of incubation.[238] A single metabolite (a substituted pyridine carboxylic acid) accounted for about 60% of the fluridone added. Persistence of nonflurazon in soil was variable with half-lives ranging from 70 to 270 d[239] although damage to sensitive plants was observed up to 13 months after application.[240,241] Persistence of nonfluorazon in soil appeared to be a function of attenuation by organic colloids.[235,240]

Within 54 d after application, triclopyr was converted to two major metabolites (3,5,6-trichloro-2-pyridinol, and 2-methoxy-3,5,6-trichloropyridine) which accounted for 85 and 10%, respectively, of the triclopyr added. Both degradation products resulted from oxidation of the acetic acid moiety at position 2 of the pyridine ring.[142] Imazethapyr exhibited biphasic dissipation in soil, where dissipation was rapid in the first 30 to 45 d and the remaining herbicide became resistant to dissipation.[242] In field studies, persistence ranged from a few months in low

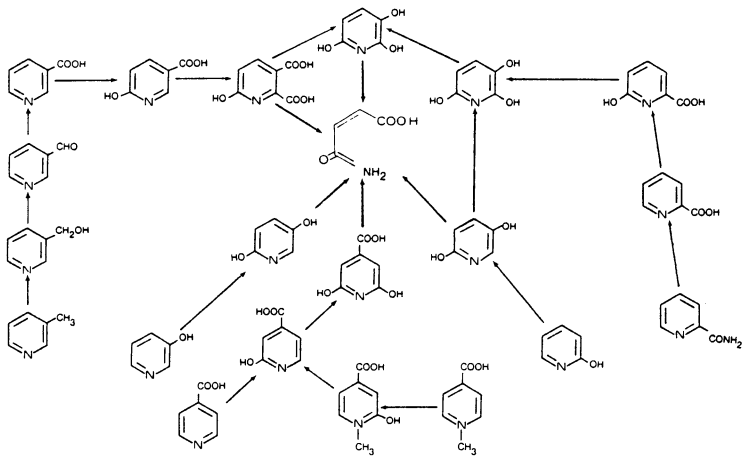

FIGURE 18. Convergence of biodegradation mechanisms for a diverse collection of pyridines into a central maleamate pathway.

organic matter, coarse textured soils to at least 3 years in high organic matter, fine-textured soils.[242] In laboratory studies, imazethapyr dissipation was the result of degradation by microorganisms.[243] The rate of degradation under laboratory conditions appeared to be determined by the bioavailability of herbicide to microorganisms.[244]

Four-aminopyridine has been used as an avicide and chemical frightening agent for birds. Betts et al.[245] reported a relatively long half life (less than 25% was degraded during the 60-d duration of the experiment) for the compound in soils. This result was consistent with previous and subsequent findings which suggest that aminopyridines are persistent.[101,121,150] Starr and Cunningham[246] reported accumulation of metabolites (unidentified) from 4-aminopyridine in soil.

VII. CONCLUSIONS

Pyridine derivatives comprise a large and important class of environmental contaminants. Most environmentally significant representatives of this class are moderately to highly soluble, and are therefore expected to be susceptible to transport, thus posing a threat of local surface or groundwater pollution. Such contamination has been verified directly through monitoring of wells and waterways proximate to sources of contamination. Mechanisms exist for photolysis, volatilization, complexation, surface attenuation, and biodegradation among pyridines which have been detected in the environment. Most of these data have been obtained under somewhat artificial conditions, and probably should be validated by experiments in a more natural setting.

Reactivity of pyridines is strongly influenced by the nature and position of ring substituents, therefore general statements regarding their environmental fate must either be precedented by the chemistry of the particular species of interest, or based upon direct experimental evidence.

It is clear from the available literature that biological degradation constitutes a major mechanism for detoxification or dissipation of select pyridines from the environment. A substantial quantity of research data has contributed to our understanding of the mechanisms available for catabolism of pyridine rings, although reactions peculiar to this class of compounds (such as aerobic ring reduction) remain poorly understood. Best understood are pathways involving initial hydroxylation with oxygen derived from water, and subsequent convergence of diols or triols into the maleamate pathway (Figure 18). Perhaps least understood are the mechanisms for catabolism of alkyl- and chloropyridines, which constitute two of the largest classes of pyridines occurring in the environment. Moreover, the total picture of the environmental fate of most pyridines which have been detected in surface and groundwater samples remains obsure. Future research should be directed toward answering these questions.

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